peptidoglycan (PG), a biopolymer forming a multi-gigadalton bag-like structure, and additionally in Gram-positive bacteria, of covalently linked anionic polymers called wall teichoic acids (WTA).
The machinery involved in the synthesis of this envelop is crucial and is one of the main antibiotic target. Different protein as transpeptidase, activator or hydrolase are recruited to maintain it morphogenesis during the bacterial cell cycle. Based on few examples involved in the machinery of synthesis of the peptidoglycan, we will demonstrates that a combination of liquid and solid-state NMR can be a powerful tool to screen for cell-wall interacting proteins in vitro and on cell.[1-2]
In particular, structure of the L,D-transpeptidases that results in b-lactam resistance in M. tuberculosis, has been studied in presence of the bacterial cell wall and in presence of antibiotic.
In parallel, we have investigated the potential of Dynamic Nuclear Polarization (DNP) to study cell surface directly in intact cells.[3] Our results show that increase in sensitivity can be obtained together with the possibility of enhancing specifically cell-wall signals.
References:
1) Egan et al. (2014). Outer-membrane lipoprotein LpoB spans the periplasm to stimulate the peptidoglycan synthase PBP1B. PNAS 111, 8197-8202.
2) Lecoq et al. (2013). Structure of Enterococcus faecium l,d-transpeptidase acylated by ertapenem provides insight into the inactivation mechanism. ACS Chem Biol 8, 1140-1146.
3) Takahashi et al. (2013) Solid-State NMR on Bacterial Cells: Selective Cell Wall Signal Enhancement and Resolution Improvement using Dynamic Nuclear Polarization. J Am Chem Soc.

216-Wkshp
Solid-State NMR Study of Intact Microalgae
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The study of intact cells by solid-state NMR is challenging considering their high level of complexity. Therefore interactions with the lipid membranes for example have been traditionally studied using model cell membranes composed of a few representative lipids. Microalgae are excellent examples of the complexity of microorganisms. Their lipid-rich plasma membrane is, in most species, protected by a cell wall, and the cytoplasm contains a variety of organelles including the chloroplast and the nucleus, as well as storage lipids and sugars. Microalgal cell walls are diverse and can be made of cellulose, proteins, polysaccharides, silica or calcium carbonate. The diversity of lipids is also notable in terms of the headgroup variety encountered as well as degrees of unsaturation. Because microalgae are at the basis of the aquatic food chain, we are developing approaches to study the interaction of contaminants with these cells by solid-state NMR using magic-angle spinning (MAS) for maximum resolution. We have first established a protocol of 13C enrichment, enabling the acquisition of signal from all the constituents. Freshwater C. reinhardtii and marine water species P. lutheri and N. galbana have thus been 13C-labeled. The use of dynamic filters to evidence particular constituents will be discussed, such as experiments based on through-space (cross polarization) or through-bond (RINEPT) magnetization transfer to study rigid and dynamic components, respectively. Isotopic labeling strategies will also be discussed as well as the challenges to study interactions with the lipid membranes and cell wall.

Workshop: Artificial Cells: Understanding and Engineering

217-Wkshp
The Engineering of Artificial Cellular Systems using Synthetic Biology Approaches
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Artificial cellular systems are minimal systems that mimic certain properties of natural cells, including signaling pathways, membranes, and metabolic pathways. These artificial cells (or protocells) can be constructed following synthetic biology approaches by assembling biomembranes (the shell), synthetic gene circuits (the information), and cell-free expression systems (the engine). Specifically, we created new synthetic molecular systems to control genetic circuits of artificial cells. We also implemented a synthetic bacterial consortium for the synthesis of cell-free systems. Furthermore, we designed and tested a novel microfluidic platform for making uniform artificial cells. Since artificial cells are built from bottom-up using minimal and a defined number of components, they are more amenable to predictive mathematical modeling and engineered controls when compared to natural cells. Along this line, we will discuss the applications of artificial cells as drug delivery and in situ protein expression systems. Furthermore, we will discuss potential applications of artificial cells as biomimetic systems to unveil new insights into functions of natural cells, which are otherwise difficult to investigate due to their inherent complexity. It is our vision that the development of artificial cells will bring forth parallel advancements in synthetic biology, cell-free systems, and in vitro systems biology.

218-Wkshp
Engineering Synthetic Ribosomes In Vitro
Michael Jewett.
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Purely in vitro ribosome synthesis could provide a critical step towards unraveling the systems biology of ribosome biogenesis, constructing minimal cells from defined components, and engineering ribosomes with new functions. To that end, we have developed an integrated synthesis, assembly, and translation technology (termed iSAT) for the in vitro construction of Escherichia coli ribosomes in crude ribosome-free S150 extracts. iSAT allows for the simultaneous in vitro synthesis of ribosomal RNA (rRNA), assembly of rRNA with purified ribosomal proteins, and transcription and translation of a reporter protein as a measure of ribosome activity. Here, we describe the development of, and recent improvements to, the iSAT system. Key breakthroughs include refining extract preparation, tuning the transcriptional balance of rRNA and mRNA production, and adding crowding and reducing agents. In addition to technological advances, we show that iSAT makes possible the in vitro construction of modified ribosomes by introducing a 23S rRNA mutation that mediates resistance against clindamycin. We also show that iSAT can be used for studying ribosome assembly. We anticipate that iSAT will aid studies of ribosome biogenesis and the construction of artificial cells.

219-Wkshp
Measuring Gene Expression in Fly Embryos: From Single Molecules to Network Dynamics
Thomas Gregor.
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The nodes of many genetic networks that are active during early development are transcription factors, i.e. proteins that cross-regulate each other via activating or repressive interactions. Hence, in order to understand generic properties of such transcription networks, obtaining quantitative access to the molecular players is key. In particular, in addition to proteins, quantitative handles to other molecular species such as RNA-polymerases and mRNA molecules are crucial to understand the transition from one network node to the next. I will report on our recent progress in developing methods to count individual molecules of mRNA in intact fly embryos, and to monitor in vivo the transcriptional activity of nascent mRNA at their site of production on the DNA. Initial applications and results using these methods will also be discussed.

220-Wkshp
Evolution Experiment With Translation-Coupled RNA Replication System
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The ability to evolve is a key characteristic that distinguishes living things from non-living chemical compounds. The construction of an evolvable cell-like system from non-living molecules has been a major challenge. We encapsulated an artificial RNA genome and the factors for protein synthesis into water droplets in oil or lipid vesicles to develop an evolvable artificial cell model. In the micro-compartments, the artificial genomic RNA replicates through the translation of its encoding RNA replicase gene. Using the translation-coupled RNA replication system, we performed a long-term (600-generation) evolution experiment, in which mutations were spontaneously introduced by the translated replicase into its genetic information. At the beginning, the amplified RNA genomes were in the double stranded form, a dead-end product for the translation while a small parasitic RNA appeared by a deletion mutation on the RNA genome and dominated the population by stealing the replicase translated from the RNA genome. But highly replicable mutant RNA genomes gradually evolved to eliminate the two short circuits by reinforcing the interaction with the translated replicase. At the end, two-order acceleration in replication rate was observed whereas the population declined when the same reaction was conducted in a bulk solution. The results indicated that the micro-compartmentalization was essential for the assembly of the bio-polymers to evolve.